MITOSIS IN HYDRA

Mitosis in the Ectodermal Epithelio-Muscular Cells of Hydra

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Introduction and Historical

Various workers with *Hydra* have come to the conclusion that the ecto-epithelio-muscular cells of *Hydra* do not divide, and that if mitotic figures are found in them it is the exception rather than the rule. Downing (1905) says, "In all the sections studied mitosis has been the universal mode of division in the interstitial cells, the exceptional mode in the ectoderm cells and amitosis the constant rule in the endoderm cells. Fig. 5 shows an ectoderm cell in process of amitotic division. At first it was thought this was the only mode of division of the ectoderm cells. But a few cases have been observed, only two or three in hundreds of sections, however, where mitosis occurred in an undoubted ectoderm cell. Schneider had observed and figured mitosis in an ectoderm cell. But cell multiplication, except of the interstitials seems to be almost invariably amitotic." (Pages 411–412.)

Strelin (1928) says, "K. C. SCHNEIDER (1890) findet in seiner eingehenden zytologischen Untersuchung gar keine Übergangszellenformen zwischen den I. Z. einerseits und den Epithelmuskelzellen andererseits; deshalb ist er geneigt zu denken, das die Neubildung der Epithelmuskelzellen auf Kosten der Teilung der differenzierten Elemente stattfindet. Als Bestätigung führt SCHNEIDER die Teilungsbilder der Epithelmuskelzellen an.

"Vielen Autoren ist es aber gar nicht gelungen, in den spezialisierten Zellen der Hydra Mitosen zu beobachten; ich konnte ebenfalls in diesen Zellen kein einziges Mal Mitosen beobachten, obgleich ich zu diesem Zwecke eine grosse Zahl von Schnitten durchgesehen habe. SCHNEIDER (1890), STECHE (1911), GELEI (1924), MATTES (1925), KANAJEIO (1926) u.a. haben in den zpezialisierten Zellen Mitosen angetroffen, sie halten aber dieselben für eine seltene Erscheinung. Es ist daher kaum möglich, die Neubildung der Epithelzellen ausschliesslich durch die Vermehrung derselben zu erklären." (Page 274.)

We see then that Strelin maintains that the indifferent cells are transformed into ecto-epithelio-muscular cells in mitosis. He further states, "Die Frage darüber, auf welche Weise aus einer ganzen Gruppe kleiner junger Epithelzellen nur mehrere Elemente entstehen, ist für mich nicht ganz klar, um so mehr, als die Zahl der Zellen sich stets durch Vermehrung vergrössern muss. Es ist möglich, dass im gegebenen Falle die Rückentwicklung der jungen Epithelzellen in Interstitiellzellen stattfindet; es ist aber viel wahrscheinlicher, dass einige junge Epithelzellen auf den Anfangsstadien ihrer Entwicklung aufgehalten werden. In den grösseren von den jungen Epithelzellen kommen ebenfalls, wie oben vermerkt wurde, Mitosen vor; man muss aber bemerken, dass je mehr die Zellen differenziert sind, desto seltener in ihnen Mitosen vorkommen; in den erwachsenen Epithelzellen ist es mir niemals gelungen, wie ich bereits erwähnt habe, Mitosen zu begegnen." (Pages 278–279.) We see further, that Strelin noticed the difference in the size of the indifferent cells, but he calls the larger of these, which he found in mitosis, "jungen Epithelzellen" and thinks that when these "jungen Epithelzellen" are further transformed into ecto-epithelio-muscular cells they never divide.

McConnell (1930 and 1932) has demonstrated the mitosis of the endo-epithelio-muscular cells and made reference to the mitosis of the ecto-epithelio-muscular cells. Owing to the fact that so many mitotic figures have been found (and easily found) in the ecto-epithelio-muscular cells, it was decided to make a study of this process to clarify the question of mitosis in these cells.

Materials and Methods

Pelmatohydra oligactis was used in this study. The material was collected in the vicinity of the University of Virginia, Charlottesville, Virginia, U. S. A.; the University of Ljubljana, Ljubljana, Jugoslavia; the Kaiser Wilhelm-Institute for Biology, Berlin-Dahlem, Germany. Preparations studied were as follows: without buds 105, with buds 75, with testes 15, with ovaries and testes 7, with ovaries 3. These *Hydra* were killed and fixed in the following solutions: in Bouin's, in Fleming's, in Carnoy's acetic alcohol, and in Zenker's. Sections were cut at five and seven microns. Staining was done on the slide with iron-hematoxylin and acid fuchsin, with Delafield's hematoxylin, and Bordeaux red; methyl violet and water-soluble eosin were also used in some instances. The preparations were mounted in Damar and Canada Balsam. They were studied with a compound binocular microscope equipped with 430 × dry and 97 × oil immersion objectives; 5 ×, 10 ×, and 15 × paired oculars were used. Microphotographs were made at magnifications of 430 ×, 860 ×, and 970 ×, during which processes an Orsam point-light was used.

Prophase

During the prophase the nucleus enlarges and moves slightly toward a central distal position; it takes nuclear stains much more intensely than do the normal resting nuclei. (See Fig. 3, Plate I.) The number of nucleoli varies from one to four, and they remain evident up until the beaded spireme is formed. The entire cell rounds up and the muscular processes are withdrawn from the mesoglea. This rounding-up process gives a characteristic swollen appearance to the area where the mitosis is taking place. The beaded spireme is very active in the nuclear area and may be seen, in maceration preparations, to be very actively whirling about. There are twelve chromosomes of V-shape.

Metaphase

The chromosomes line up on the equatorial plate with the tips of the V's pointed to the center. The asters and centrioles are quite prominent. The chromosomes start dividing at the tip of the V, and continue dividing longitudinally. As the open arms of the daughter chromosomes recede, they remain connected by spindle fibers.

Anaphase

As the daughter chromosomes approach the division centers the tips of the V's fuse slightly, with the ends of the daughter chromosomes protruding in various directions. As they move towards the pole the centriole becomes more and more indistinct and gradually disappears.

Plate I

1. Resting nucleus with four nucleoli. Bouin's; iron-hematoxylin. 776 ×.

2. Early prophase to the left, and daughter cell to the right. Bouin's; iron-hematoxylin, and acid fuchsin. 776 ×.

3. Lower left, normal resting nucleus; upper right, later stage of prophase. Bouin's; iron-hematoxylin and acid fuchsin. 776 ×.

4. Prophase just prior to the disappearance of nuclear membrane. Bouin's; iron-hematoxylin. 776 ×.

5. Same as No. 4. Bouin's; iron-hematoxylin and acid fuchsin. 776 ×.

6. The metaphase chromosomes. Bouin's; iron-hematoxylin. 776 ×.

7 and 8. Two metaphase stages. Notice the slight bulge which makes this stage very noticeable. Bouin's; iron-hematoxylin and acid fuchsin. 776 ×.

9. Metaphase from the base of tentacle. Bouin's; iron-hematoxylin and acid fuchsin. 776 ×.

10. Metaphase. Fleming's; iron-hematoxylin and acid fuchsin. 776 ×.

11. Metaphase. Carnoy's acetic alcohol; iron-hematoxylin and acid fuchsin. 776 ×.

12. Metaphase perpendicular to mesoglea. Zenker's; iron-hematoxylin and acid fuchsin. 776 ×.

PLATE I

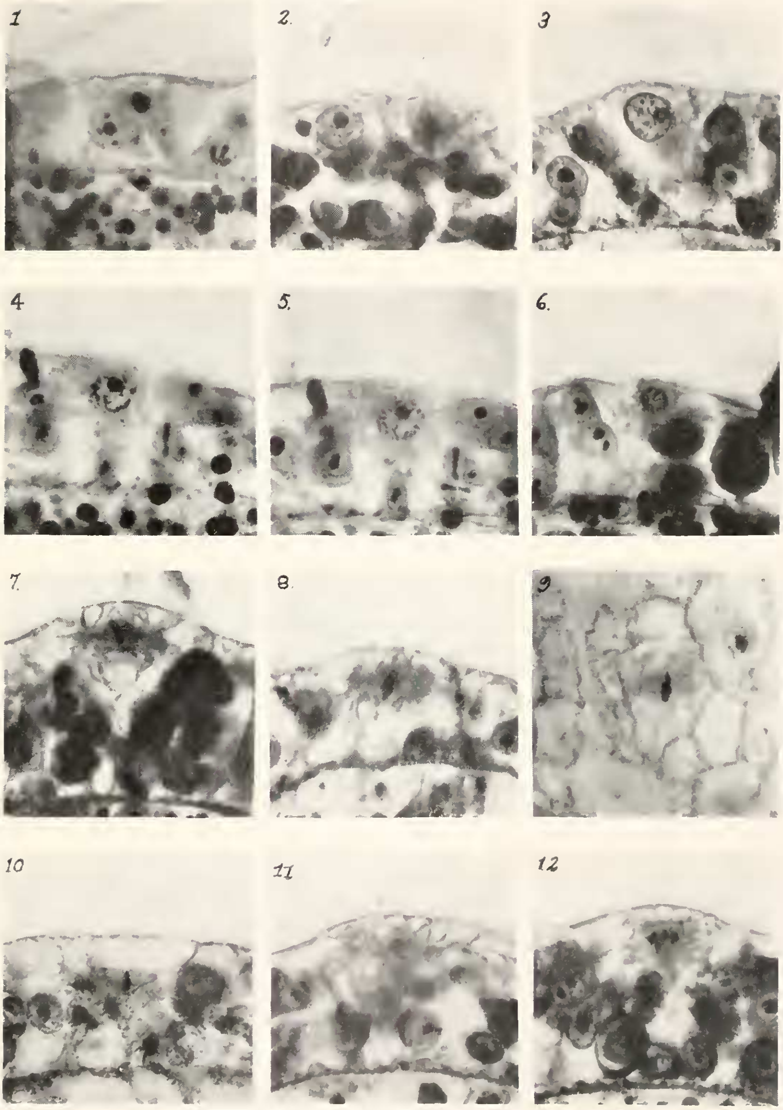
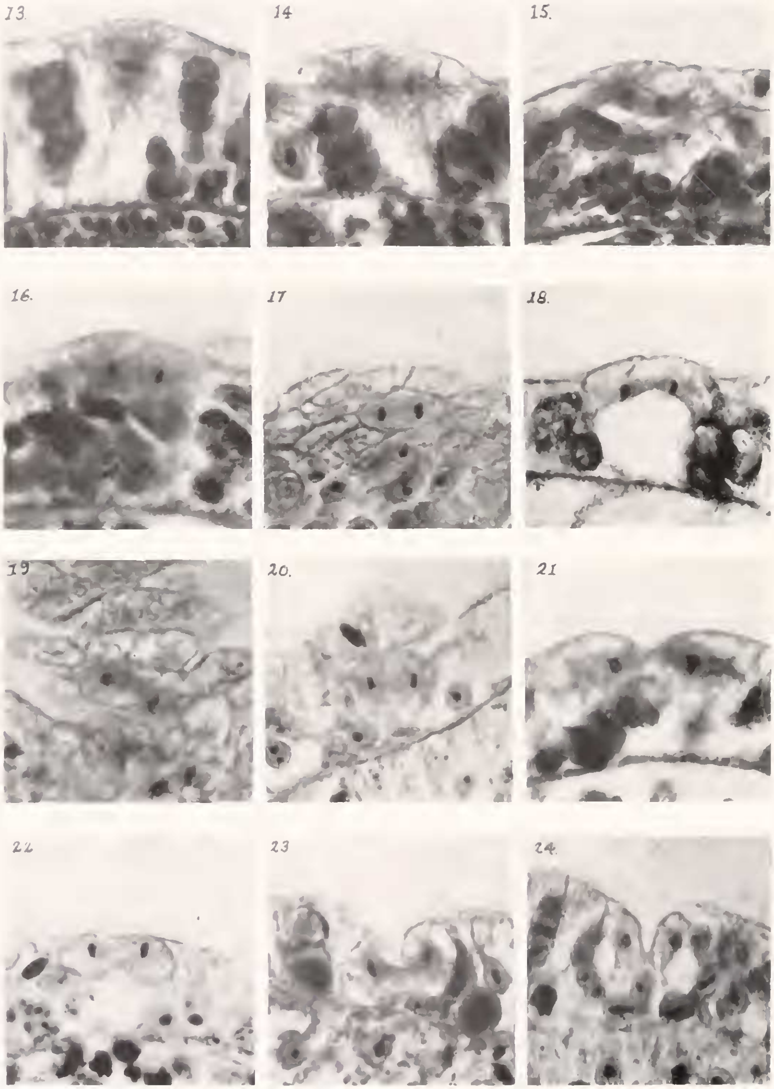


Plate II



Telophase

Upon reaching the area where daughter nuclei will be formed the chromosomes expand very quickly and reconstruct nuclei. The cleavage furrow is most noticeable in mitotic figures in areas where the cells are not closely crowded together or when the animal has been killed and fixed in an expanded condition. After the daughter cells are separated and the nuclei are in the process of being reconstructed,

Table I

	No. of preparations	Prophase		Metaphase		Anaphase		Telophase	
		early	late	early	late	early	late	early	late
With ovaries and testes	7	12	13	11	10	2	4	9	6
With ovaries	3	4	9	17	12	6	2	14	27
With testes	15	42	17	31	19	4	9	20	14
With buds	75	92	27	46	24	4	17	29	34
Without buds	105	101	47	30	21	7	27	49	10
Total	205	251	113	135	86	23	59	121	91

that part of the cell which lies nearest the mesoglea begins to push its way down between the surrounding cells toward the mesoglea; and, when this is reached, forms muscular processes.

Occurrence of Mitosis

As stated above, two hundred and five preparations were studied; Table I, showing the different stages, would seem to indicate that mitosis is the rule rather than the exception. Thus we find a total of 879 mitotic figures in 205 preparations.

Plate II

13. Metaphase perpendicular to mesoglea. (Compare with No. 7, Plate I.) Bouin's; iron-hematoxylin and acid fuchsin. 776 ×.
14. Anaphase. Fleming's; Delafield's hematoxylin. 776 ×.
15. Anaphase. Carnoy's acetic alcohol; iron-hematoxylin and acid fuchsin. 776 ×.
16. Anaphase. Bouin's; iron-hematoxylin and acid fuchsin. 776 ×.
17. Anaphase. Fleming's; iron-hematoxylin. 688 ×.
18. Anaphase. Note large vacuole between achromatic figure and mesoglea. Bouin's; iron-hematoxylin and acid fuchsin. 776 ×.
19. Anaphase from base of tentacle. Bouin's; Delafield's hematoxylin. 776 ×.
20. Anaphase. Bouin's; iron-hematoxylin. 688 ×.
21. Telophase. Bouin's; iron-hematoxylin and acid fuchsin. 776 ×.
22. Late anaphase. Fleming's; iron-hematoxylin. 688 ×.
23. Telophase. Zenker's; Delafield's hematoxylin. 776 ×.
24. Telophase from tip of growing bud. Fleming's; iron-hematoxylin and acid fuchsin. 776 ×.

These mitotic figures are found in all parts of the *Hydra's* body except in the epithelio-muscular cells of the tentacles. The number of mitotic figures varies greatly as to the region of the body; the majority are found in all cases in the upper two-thirds of the body; however, many may be found in the so-called "stalk-region" and on the peristome. These dividing cells may also be found in the basal disc, but this appears to be rather rare.

In the majority of cases, the achromatic figure during the metaphase is parallel to the mesoglea; however, in some exceptional cases, one finds the metaphase spindle arranged perpendicularly to the mesoglea, in which case one of the daughter cells is buried under the surrounding epithelial cells; the fate of these buried cells has, as yet, not been determined. (Fig. 12, Plate I, Fig. 13, Plate II, and Fig. 36, Plate III.)

Discussion

Kanajew (1930), who worked quite extensively on the problem of regeneration in *Hydra*, and who reviewed all of the literature on regeneration, says: "Mitosen sind nur in den I-Zellen häufig. In differenzierten Ekto- und Endodermzellen habe nur seltene Falle der Zellvermehrung bemerkt." Since Kanajew was working on regenerating *Hydra* and not *Hydra* in their normal condition, it is quite natural that he should not find many cases of the mitosis of the ecto- and endo-epithelio-muscular cells. The indifferent interstitial cells, as he noted, are found in mitosis in *Hydra* under all conditions of experimentation.

Threlkeld and Hall (1928), in their experiments upon *Hydra* under determined ion concentration, starved *Hydra* over long periods of time.

Plate III

25. Telophase. Zenker's; iron-hematoxylin. 776 ×.

26. Telophase. Carnoy's acetic alcohol; Delafield's hematoxylin. 776 ×.

27. Telophase from tip of bud. Note remains of spindle fibers. Bouin's; iron-hematoxylin. 688 ×.

28. Telophase. Note remains of spindle fibers. Bouin's; Delafield's hematoxylin. 688 ×.

29. Late telophase, from peristomal region. Bouin's; iron-hematoxylin and acid fuchsin. 776 ×.

30, 31, and 32. Stages at which the daughter cells begin growing down toward the mesoglea, where muscular processes will be formed. Bouin's; Delafield's hematoxylin and Bordeaux red. 776 ×.

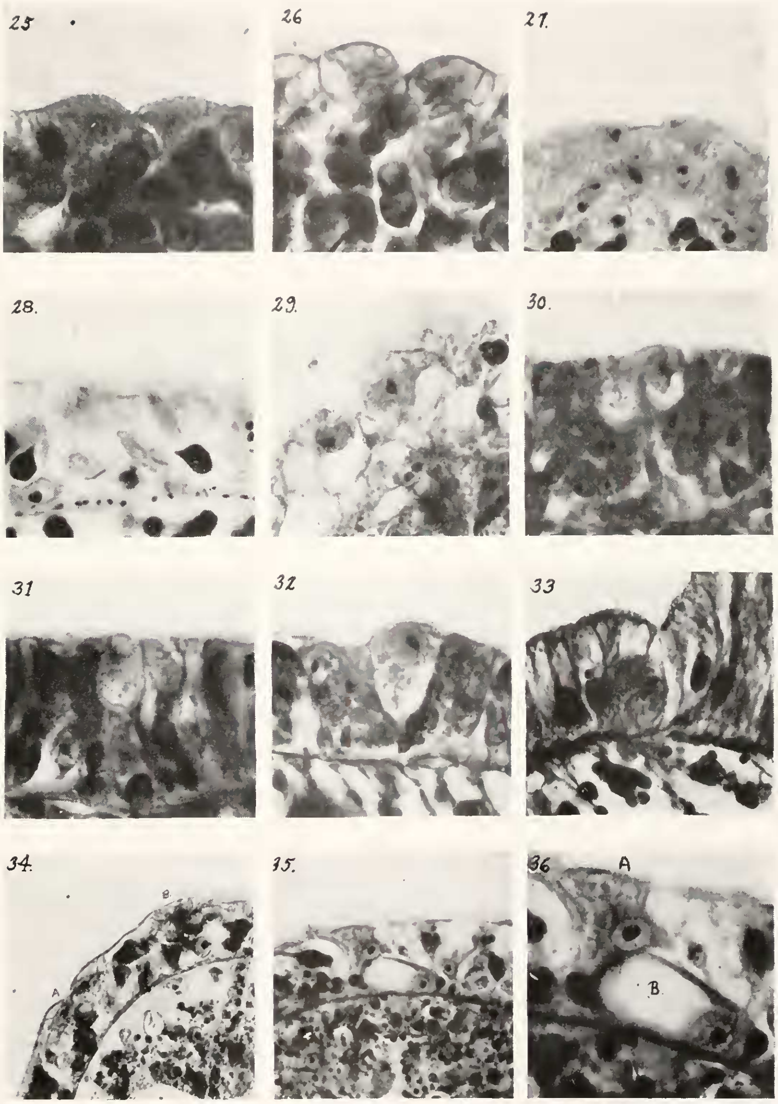
33. The two daughter cells resulting from the division of one of the mucus-secreting cells of the basal disc. Bouin's; iron-hematoxylin and acid fuchsin. 776 ×.

34. A cross-section through the lower third of a *Hydra*'s body, showing a telophase at *A*, and a metaphase at *B*. Bouin's; iron-hematoxylin and acid fuchsin. 344 ×.

35. The result of division of an epithelio-muscular cell when the metaphase was not parallel to the mesoglea. Bouin's; iron-hematoxylin. 344 ×.

36. Same as No. 35. The buried cell, *B*, has arisen from the cell *A*. 776 ×.

PLATE III



A close study of one of these preparations (*Hydra* starved 19 days), loaned to me by one of the authors, revealed the following figures given in Table II.

TABLE II

	Prophase	Metaphase	Anaphase	Telophase
Ecto-indifferent	5	8	3	1
Secretory cells	1	1	0	0
Endo-epithelio-muscular	0	0	0	0
Endo-indifferent	0	0	0	0
Ecto-epithelio-muscular	2	1	0	2

We see from Table II then that the division of the cells of *Hydra* may take place under the most adverse circumstances, namely, reduction and resorption, and starvation.

It appears from all the material studied that mitosis is a normal process in the ecto- and endo-epithelio-muscular cells of *Hydra*. It must be admitted that occasionally one finds nuclei which might give the appearance of having arisen by amitosis; but these cases are so rare and so difficult to account for, that they can best be omitted in the present discussion. It may be noted in passing however, that these, which appear to be amitotic divisions, sometimes occur side by side with cells which are going through the process of mitotic division.

If we assumed that the epithelio-muscular cells did not divide but were eventually replaced by cells which were elaborated from the indifferent cells, we should constantly find the indifferent cells of the ectoderm in the rather constant process of enlarging to almost four times their normal size; developing a muscular process with a myoneme; and developing large vacuoles, all of which are characteristic of the ecto-epithelio-muscular cells. If we assumed that the endo-epithelio-muscular cells did not divide we should find the various stages of the endo-interstitial cells in which they are developing muscular processes, food vacuoles, and flagella; but this is not true of any of the material studied so far, for at no time were there found any indifferent cells which might even be mistaken or described as assuming any of the foregoing characteristics of the endo- or ecto-epithelio-muscular cells. It seems then that these endo- and ecto-epithelio-muscular cells are self-propagating by the process of mitosis and that the endo- and ecto-indifferent cells are concerned not with replacing them, but with other processes, namely, with the formation of sperm, eggs, nematocysts, and nerve net cells of the ectoderm; and the formation of nerve cells, secretory cells of the general endoderm, and peristomal gland cells of the endo-peristomal region.

Summary

1. The ecto-epithelio-muscular cells of *Hydra* divide by mitosis.
2. A centriole and asters are present. There are 12 V-shaped chromosomes.
3. These mitotic divisions take place in *Hydra* under all conditions.
4. There is no evidence that the indifferent interstitial cells of the ectoderm or endoderm are elaborated into the large epithelio-muscular cells.

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